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Reproductive cycle of *Arvicola terrestris* (Rodentia, Arvicolidae) in the Aran Valley, Spain

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Abstract

Studied the reproductive biology of *Arvicola terrestris* in the Aran Valley (Lérida, Spain). 684 specimens (304 males, 380 females) captured between July 1983 and December 1984 were analysed. The breeding season of this population starts in March and ends in October/November. Spermatozoids are present in adult individuals throughout the year, although the testicles and seminal vesicles vary in size. The minimum size is reached in December and the maximum between April and June. During the breeding season, males attain sexual maturity before females. The sexual activity in mature females varies throughout the year with a maximum between May and August/September. Pregnant females are found between March and October/November. The average litter size is 4.48 ± 1.23, n = 80.

Introduction

Although the data published on the reproduction of the northern water vole, *Arvicola terrestris* are abundant (Reichstein 1982), studies of a complete annual cycle are relatively scarce (van Wijngaarden 1954; Pelikán 1972; Wieland 1973; Morel 1981). Apart from some data given by Gosálbez (1976), there is no detailed information on the reproductive biology of *A. terrestris* from Spain. The purpose of this paper is to indicate the reproductive characteristics of this species in the northeast of the Iberian Peninsula.

Material and methods

The analysed specimens (n = 684; 304 males, 380 females) originate from monthly captures between July 1983 and December 1984 in the meadow lands on the left shore of the Garonne river between the localities of Aubert (930 m) and Arròs (900 m), in the Aran Valley (Lérida, Spain). The animals were collected after death and subsequently dissected to study their sexual state.

The body weight of each specimen was taken. To determine the stage of maturity and sexual activity in males, the following characteristics and parameters were considered: position of testicles (abdominal or scrotal), testicular cell content (Gosálbez et al. 1979), minor and major diameters of the testicle and length of the seminal vesicle (Gosálbez and Sanscoma 1976).

Three categories of males were distinguished from the testicular cell content, according to the criteria mentioned by Ventura and Gosálbez (1987): immature: specimens lacking spermatids and spermatozoids in the testicle; submature: specimens with few spermatozoids and spermatids in the testicle; mature: specimens with a large amount of spermatozoids in the testicle.

In the females, the main criterion used to determine the stage of maturity was the histological appearance of the ovaries (Ventura et al. 1989). Immediately after capturing the animal, ovaries were extirpated and kept in the fixation liquid (Bouin). Once in the laboratory, conventional histological procedures were carried out: embedding in paraffin, preparation of 5–7 μm thick sections stained with hematoxylin-eosin.

The following characteristics were also studied: status of the vulva (open or closed), stage of development and vascularization of the uterus and ovaries, and presence and number of embryos and placental scars.

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The following categories were established:

Immature: species not yet in the first oestral cycle (corpora lutea lacking in the ovary). They present closed vulva, poorly developed and vascularized uterus and lack placental scars.

Inactive mature: specimens displaying at least one ovulation (corpora lutea in the ovary). They present closed vulvae. The uterus is developed but scantily vascularized. They may present placental scars but no embryos.

Active mature: specimens displaying at least one ovulation (corpora lutea in the ovary). They present an open vulva. The uterus is fully developed and well vascularized. They may present placental scars and/or embryos.

To determine the average weight at which individuals reach sexual maturity, W_{50} was calculated. This estimate represents the body weight at which 50 % of the specimens are mature. This weight is the limit above which all individuals are considered adults (PELIKÁN 1972). W_{50} has been calculated according to the method described by LESLIE et al. (1945) and taking into account the presence of spermatozoids in males and of corpora lutea in females.

All the specimens were distributed into six classes of relative age (0–V) according to their type of coat, stage of moult (MAXIMOV 1959; MOREL 1981) and characteristics of the skull (KRATOCHVÍL 1974). The diagnostic traits for skull were (VENTURA 1988): degree of flatness of skull case, development of the mastoid process and separation of the interorbitary crests. In the mandible, the degree of development of the condyloid tuberosity and the separation of the angular process in relation to the base of the condyloid branch.

The intervals of age and the characteristics of the coats and moults corresponding to each class are the following (VENTURA 1988):

Class 0: 0–3 weeks. Specimens with the first coat and lacking melanic prints.

Class I: 3–6 weeks. Specimens with the first coat partially or totally developed and with regular prints corresponding to the ventral sequence of the first moult.

Class II: 6–10 weeks. Specimens with melanic prints corresponding to the dorsal sequence of the first moult.

Class III: 10–14 weeks. Specimens with the second coat partially or totally developed and with melanic prints corresponding to the second moult.

Class IV: older than 14 weeks, but before the end of their first winter. Specimens with adult coat. Irregular melanic prints may be present.

Class V: specimens that have wintered at least once. They have adult coat. Irregular and scattered melanic prints may be present.

Results and discussion

Reproduction in males

Fig. 1 reveals that starting at a certain body weight spermatozoids are present throughout the year, although their density undergoes seasonal variations. During December and January some specimens with few spermatozoids in correlation to their body weights are observed.

According to these observations, the November–February period has not been taken into account when determining the minimum weight of sexual maturity. During the remainder of the year, all individuals weighing less than 65 g are immature. Specimens between 65 and 95 g are at different stages of maturity, and above 95 g only mature individuals are found. The W_{50} obtained in males is 73 g (Fig. 2).

The lengths of testicles (TL) and seminal vesicles (VL) vary between 3.8–12.2 mm and 2.0–19.5 mm, respectively. Not taking into account the November–February period, spermatozoids appear in TL and VL ranging between 5.5 mm and 2.8 mm, respectively. Between 5.5–7.5 mm of TL and 2.8–7.0 mm of VL there is a noticeable variation in the stage of sexual maturity. Above these ranges, all individuals show a high density of spermatozoids in the testicular contents.

These values are clearly lower than those found in the literature. Thus, WIELAND (1973) found in Eastern Germany fertile males of *A. terrestris* with TL > 10 mm. PELIKÁN (1972) in Czechoslovakia found VL minimum values higher than 13 mm. These differences are due to larger body size of the specimens of these populations.

Those individuals captured in December and January with a low spermatozoid density in relation to their body size also show reduced TL (4.9–6.3 mm) and VL (6.1–8.4 mm).

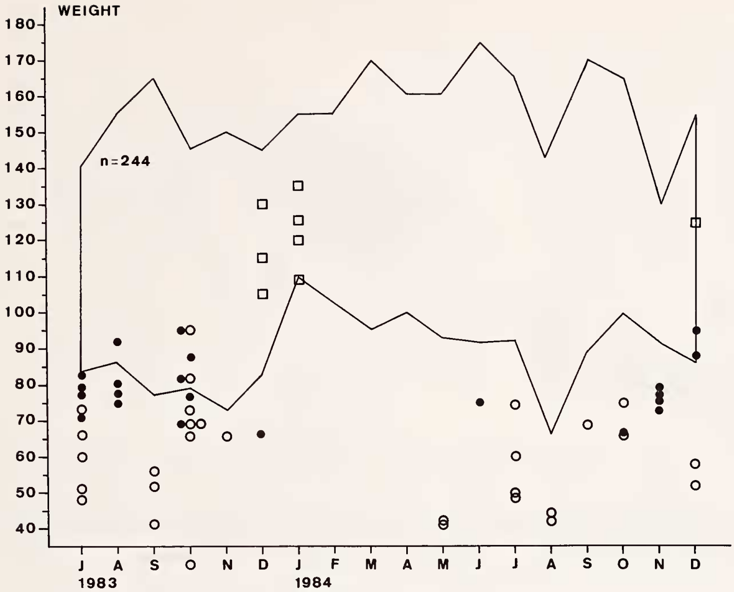

Fig. 1. Relationship between body weight (in g) and sexual status of males of *A. terrestris* from the Aran Valley, throughout the period of study. ○ = immature; ● = submature, □ = mature with few spermatozoids; solid line: remainder of mature specimens (n)

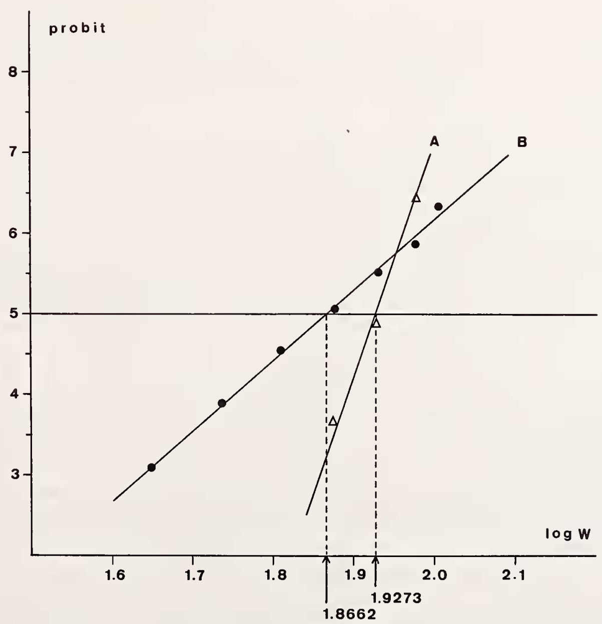

Fig. 2. Determination of 50 % of sexual maturity in *A. terrestris* from the Aran Valley. The probit corresponding to the percentage of mature specimens is plotted against the logarithm of body weight (log W). The equations for the straight lines are B: males: y = 4.9314 + 8.8188 (x − 9.8584); log (weight 50 % maturity) = 1.8662; antilog 1.8662 = 73.48 g; A: females: y = 4.8840 + 28.1679 (x − 1.9232); log (weight 50 % maturity) = 1.9273; antilog 1.9273 = 84.58 g

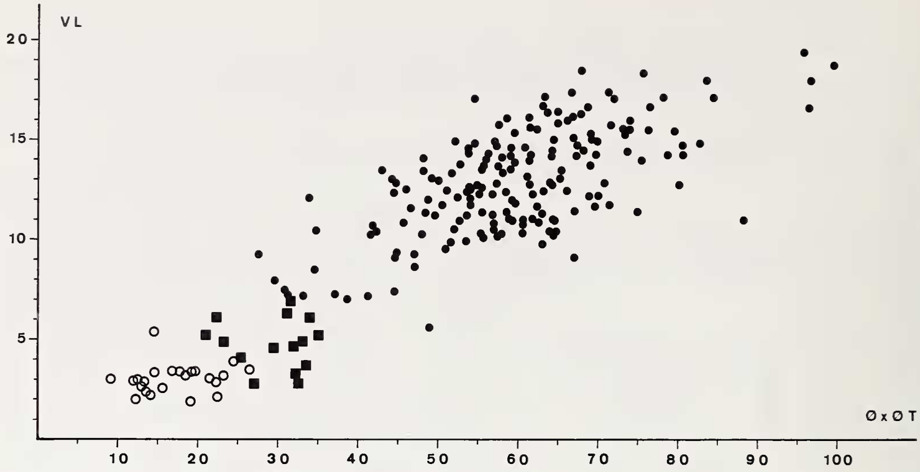

Fig. 3. Relationship between the sexual status of males and the correlation between the product of the minor and major testicular diameters (∅ × ∅ T, in mm^2) and the seminal vesicle length (VL, in mm) in *A. terrestris* from the Aran Valley. ○ = immature, ■ = submature, ● = mature

The correlation between the product of the minor and major testicular diameters (∅ × ∅ T) and VL (Fig. 3) allow us to discriminate the individuals according to their sexual stage. Only the specimens captured between March and October are taken into account. Below 25 mm^2 of ∅ × ∅ T and 4.0 mm of VL only immature specimens appear. Between these two values and 35 mm^2 and 7.0 mm, respectively, submature individuals appear, some males also being found without spermatozoids. Above these upper limits, all the specimens are sexually mature. These values are lower than those reported by Pelikán (1972) (70 m^2 ∅ × ∅ T and 9.0 mm VL), a fact due to the larger body size of *A. terrestris* in Czechoslovakia.

All males of classes 0 (n = 4) and I (n = 18) are immature. The first submature specimens are found in class II (57.14 %, n = 35). In this age class, immature (14.28 %) and mature (28.57 %) specimens also appear. In class III it is possible to find, although in small percentage, submature males (5.12 %, n = 78). These specimens were captured during the October–December period. A delay in the onset of sexual maturity in those individuals born between the end of the summer and the beginning of the autumn can be deduced. In classes IV (n = 102) and V (n = 66) only mature specimens appear. According to these results, the onset of sexual maturity in males takes place in class II.

The testicles and seminal vesicles of adult specimens (classes IV and V) undergo significant changes in size throughout the year (Fig. 4). Between June and October there is a slow but progressive decrease in the mean values of TL and VL, a process that is enhanced during November. In December and January, both parameters reach minimum values. From January on, there is an obvious size increase, which shows maximum values between April and June. A clear relationship between testicle and seminal vesicle lengths can be observed ($r = 0.90 \pm 0.04$; f.d. = 122; $p < 0.001$).

Wieland (1973) mentioned a cyclic variation of the testicle size throughout the year in *A. terrestris*. The pattern of variation that he described coincides with the observations in the Aran Valley. Likewise, Wieland (1973) indicated that the size reduction of the testicles inevitably implies sexual inactivity in males, but he did not explain precisely the causes of this alteration.

Claude (1970) and Martinet (1972) indicated that size reduction of the testicle determines a total pause or partial suppression of spermatogenesis. Therefore, the first

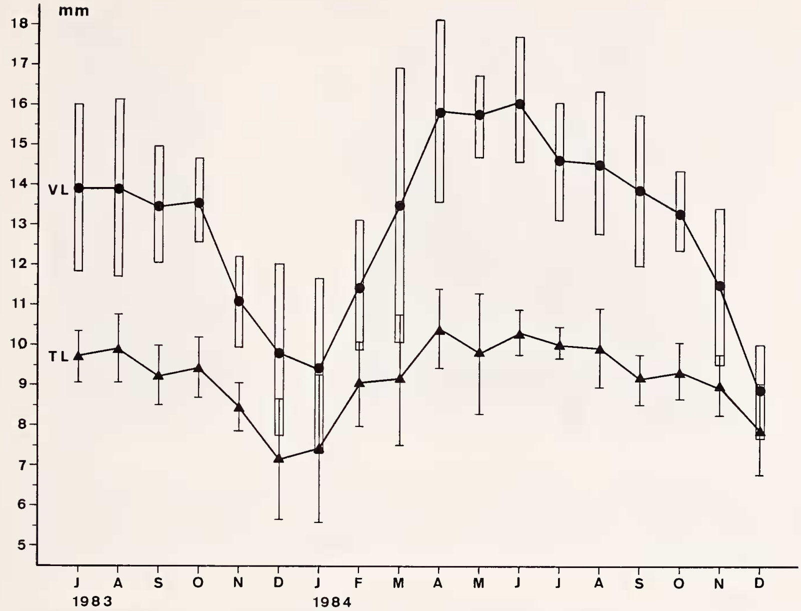

Fig. 4. Monthly variation of the average ($\bar{x} \pm s$) length of the seminal vesicle (VL) and of the testicle (TL) in adult specimens (classes IV and V) of *A. terrestris* from the Aran Valley

circumstance does not seem to have occurred in the studied population since spermatozoids in adult individuals are present throughout the year.

According to microscopic observations, the existence of a reduction in the spermatogenesis in some adult specimens during December and January can be deduced. These specimens showed especially low values of TL, even within the variation range of immature and submature males. The spermatogenic alteration seems to appear only in those mature individuals with high testicular reduction. In the remainder of the adult males, the density of spermatozoids did not show clear differences with those from individuals captured during the breeding season. However, although the adult males captured during the sexually inactive period can show a high density of spermatozoids, the testicular size reduction occurring during this period may considerably affect the reproductive potential of these individuals (Ventura and Gosálbez 1987).

Reproduction in females

Fig. 5 shows the relationship between body weight and sexual stage of females, throughout the year. During the March–October period, all specimens weighing less than 76 g are immature. Between 76 and 96 g, there is a wide range of specimens that show different stages of maturity. The lightest pregnant female weighed 86 g. All females with body weights above 96 g are sexually mature. Thus, between 76 and 96 g females reach sexual maturity.

The W_{50} obtained in females is 85 g (Fig. 2). This value is clearly higher than that found for males. Taking the body weight as an estimate of the age, the fact that males attain sexual maturity before females can be stated.

All females of classes 0 (n = 5) and I (n = 18) are immature. The first mature females appear in class II (20.41 %, n = 49). In class III the number of sexually immature individuals is still significant (15.25 %, n = 49), but in higher classes (IV = 175; V = 74) all

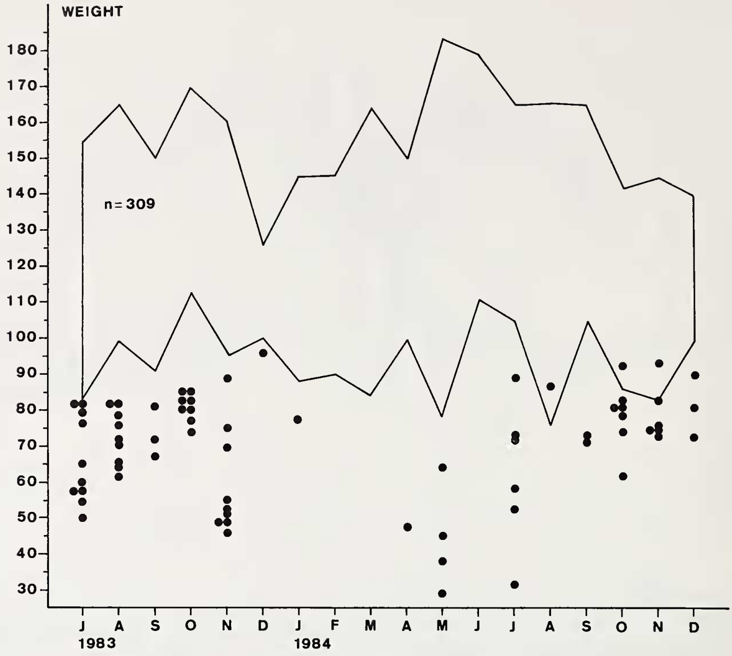

Fig. 5. Relationship between body weight (in g) and sexual status of females of *A. terrestris* from the Aran Valley, throughout the study period. ● immature, solid line: mature (n)

females have reached sexual maturity. Comparing them to the general data found for males, they seem quite coincident, although there are some particular differences. Thus, although the range of the onset of sexual maturity is also situated between classes II and III, this latter shows no immature males. These results support the observations seen previously according to W_{50}, which show that males reach sexual maturity at a lower weight than females.

The sexual stage of females according to the month of capture reveals that a relatively high percentage of the immature individuals belonging to class III (66.67 %, n = 9) were captured between November and January. This substantiates that females also show a possible delay in the onset of sexual maturity between the end of autumn and winter.

The percentage of active females referred to the total of mature females (Fig. 6) shows a cyclic variation throughout the year. Between May and August/September the level of sexual activity in females is maximum, but from September/October onward, there is a progressive decrease of activity. Between December and February the sexual activity is zero. During March this percentage shows a sharp increase lasting until May.

Pregnant females appear between March and October, despite the fact that one pregnant individual was captured during November 1983 (Fig. 6). The monthly percentage of pregnant individuals over the total of mature females shows a seasonal variation of reproductive intensity. The reproductive dynamics of the species throughout the year show a bimodal distribution with maximum values at the beginning (March) and the second half of the reproductive cycle (August). From these months onward, the relative percentage of pregnant females decreases progressively. Therefore, lower values are found in July and a total pause of reproductive activity at the end of the year.

Of 307 mature females, 80 (26.05 %) presented embryos in different stages of development. The total number of viable implanted fetuses was 359, distributed in litters of 2 to

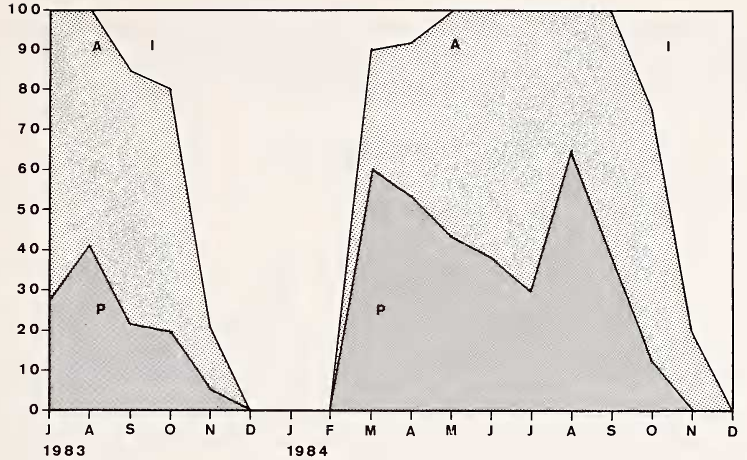

Fig. 6. Variation throughout the period of study of the percentage of active mature females with reference to the total mature females (upper line), and percentage of pregnant females over the total of active mature females (bottom line) in *A. terrestris* from the Aran Valley. I = inactive mature, A = active mature, P = pregnant

9 embryos. The average litter size is 4.48 ± 1.23 embryos/female (n = 80). This value is relatively lower than the averages registered in other populations of this species (REICHSTEIN 1982) and coincides, approximately, with the value given by GOSÁLBEZ (1976) for northeast Iberian Peninsula.

REICHSTEIN (1982) mentioned that the heaviest individuals of *A. terrestris* regularly show larger number of embryos. Likewise, PELIKÁN (1972) mentioned a geographical increase in litter size of this species from west to east of its distribution area. Regarding these observations, the two factors conditioning the low average in the analysed population could mainly be: on one hand, comparatively smaller body sizes of the individuals from the Pyrenees (VENTURA and GOSÁLBEZ 1988), and, on the other, the geographical situation of the studied population.

Conclusions

According to the present results, the breeding season of *A. terrestris* in the Aran Valley extends from March to October/November. From the monthly averages of VL and TL it is possible to conclude that the period of maximum sexual activity for males is situated between April and June. Maximum activity levels for females appear between March and August. This fact is established by the large number of pregnant females during this period.

Between November/December and February a sexual resting period appears, as all adult females are inactive and males show lower averages of LV and LT. Despite the significant size reduction of both structures during these months, all males present spermatozoids in their testicles independently of the season. Therefore, it is necessary to state that although the presence of spermatozoids allows us to classify an individual as sexually active, the reproductive efficiency needs to be verified when the size reduction of the testicle and seminal vesicle size reduction is still in progress (VENTURA and GOSÁLBEZ 1987). Despite the fact that spermatogenesis is not interrupted once sexual maturity is reached, an adult animal with reduced testicular and vesicular sizes may not be fertile. The sexual inactivity of males needs to be considered only as a secondary factor since females regulate the duration and reproductive intensity of the population (PELIKÁN 1972). Thus, the absolute inactivity of females during November/December–February determines the pause of reproductive activity, because even if males still present some fertilizing capacity (according to the presence of spermatozoids in the genital tract), the regulating effect exerted by females controls the incidence of matings.

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Zusammenfassung

Zum Fortpflanzungszyklus von Arvicola terrestris (Rodentia, Arvicolidae) im Nordosten der Iberischen Halbinsel

An 304 Männchen und 380 Weibchen von *Arvicola terrestris*, die zwischen Juli 1983 und Dezember 1984 im Aran-Tal in Spanien gefangen worden waren, wurde das Fortpflanzungsverhalten untersucht. Die Fortpflanzung beginnt im März und endet im Oktober/November. Bei den adulten Männchen findet man ganzjährig Spermatozoen, obwohl die Größe von Hoden und Vesikeldrüsen im Jahreslauf stark schwanken. Am kleinsten sind sie im Dezember, am größten von April bis Juni. Während der Fortpflanzungsperiode erreichen die Männchen früher die Geschlechtsreife als die Weibchen. Von Mai bis August/September sind alle geschlechtsreifen Weibchen auch geschlechtstätig. Geschlechtstätige Weibchen fehlen von Dezember bis Februar. Trächtige Weibchen kamen nur von März bis Oktober/November vor. Die durchschnittliche Embryonenzahl war 4.48 ± 1.23, $n = 80$.

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